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Bureau des brevets

Patent Office

Ottawa, Canada
K1A 0C9

(21) (A1)

2,042,138

(22)

1991/05/08

(43)

1992/11/09

5,024,8/77

(51) INTL.CL.⁵ A61K-031/215

(19) (CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) **Agents for Preventing or Treating Bacterial Diseases of Fishes**

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(57) 5 Claims

Notice: The specification contained herein as filed

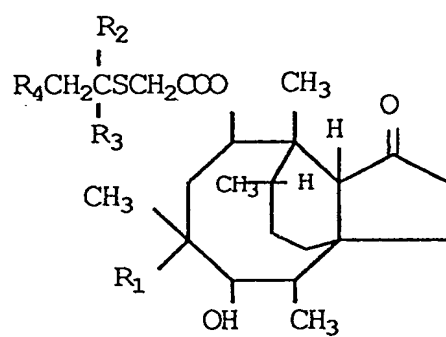
Canada

CCA 3254 (10/89) 41

ABSTRACT OF THE DISCLOSURE

The present invention relates to agents for prevention and treatment of bacterial diseases of fishes comprising, as an active ingredient, a pleuromutilin derivative.

- 5 The present invention further relates to agents for prevention and treatment of bacterial diseases of fishes comprising, as an active ingredient, tiamulin and the derivatives thereof.



SPECIFICATIONTITLE

Agents for Preventing or Treating Bacterial Diseases of
Fishes

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FIELD OF THE INVENTION

The present invention relates to agents for prevention and treatment of bacterial diseases of fishes comprising, as an active ingredient, a pleuromutilin derivative (designated
10 as the Derivative hereinafter).

The present invention further relates to agents for prevention and treatment of bacterial diseases of fishes comprising, as an active ingredient, tiamulin and the derivatives thereof.

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BACKGROUND OF THE INVENTION

The Derivatives are the known substances described in Japanese Patent L-O-P Nos. 199870/1985 and 7147/1982.

The Derivatives are semi-synthetic derivatives of
20 pleuromutilin. The precursor, pleuromutilin, is a diterpene antibiotics obtained from the cultivation filtrate of *Pleurotus multilus* by Kavanagh et al., in 1951 and the Derivatives are synthesized from the precursor pleuromutilin by chemical modification.

In recent years, cultivation of sea and fresh water fishes such as yellowtail, young yellowtail, eel, salmon, trout, sweet fish ("ayu"), carp and the like has become prosperous with the development of cultivation fishery and
5 there is a continuous demand for development of more economic cultivation technology and also safer and more effective agents for prevention and treatment of diseases.

There have been a number of precedents in which antibiotics are administered to fishes in order to inhibit
10 fish disease-causative bacteria and to prevent or treat the diseases caused by such bacteria. Many antibiotics such as oxytetracycline, ampicilin, erythromycin and so on are known as a medicament usable in the aquatic industry.

On the other hand, certain literature describes that
15 resistant strains have been frequently found in the wild strains due to an increase in the amount of commonly used antibiotics and therefore any possible means for resolving the problem has been sought.

Among many kinds of fish diseases of many kinds of
20 fishes, streptococcal infection of yellowtail is a problem to be resolved by all means in the young yellowtail cultivation which is becoming prosperous from year to year. Development of new therapy has ben demanded for such diseases in view of emergence of resistant strains. In addition, outbreaks of
25 bacterial kidney disease in fishes of Salmonidae are recently

becoming a problem, and appropriate agents for prevention and treatment of the disease are earnestly demanded.

Since the Derivatives exhibit significantly excellent antibacterial activities against the pathogen of the above-mentioned fish disease, i.e., streptococcus sp. and the pathogen of bacterial kidney disease, i.e., Renibacterium sp. without being accompanied by cross resistance between these derivatives and widely used antibiotics, they are expected to be useful as an agent for prevention and treatment of fish diseases. There have been no literature or publication describing the use of the derivatives in the prevention or treatment of fish diseases.

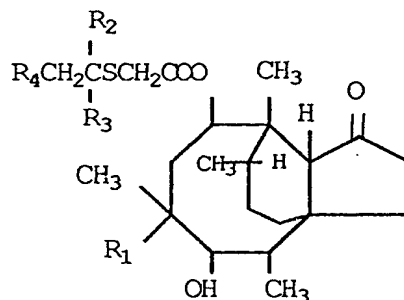
OBJECT OF THE INVENTION

The present invention has been achieved in view of the prior art mentioned above, and an object of the invention is to provide agents for prevention and treatment of bacterial diseases of fishes comprising, as an active ingredient, a pleuromutilin derivative.

A further object of the invention is to provide agents for prevention and treatment of bacterial diseases of fishes comprising, as an active ingredient, tiamulin and the derivatives thereof.

SUMMARY OF THE INVENTION

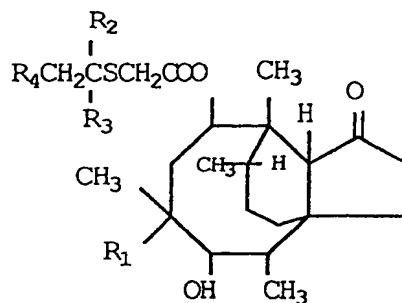
(1) An agent for the prevention or treatment of bacterial diseases of fishes according to the present invention comprises, as an active ingredient, a pleuromutilin derivative of the formula:



wherein R_1 is ethyl or vinyl, R_2 is hydrogen or methyl, R_3 is hydrogen or methyl, and R_4 is dialkylamino or R_5 -CONH- (R_5 being 5-membered saturated heterocycle or aminoalkyl which is unsubstituted or substituted with hydroxy in the alkyl moiety, in the free form or in the form of an acid addition salt or a quaternary ammonium salt).

DETAILED DESCRIPTION OF THE INVENTION

The agent for prevention and treatment of bacterial diseases of fishes according to the present invention comprises, as an active ingredient, a pleuromutilin derivative of the formula:



wherein R_1 is ethyl or vinyl, R_2 is hydrogen or methyl, R_3 is
 5 hydrogen or methyl, and R_4 is dialkylamino or R_5 -CONH- (R_5
 being 5-membered saturated heterocycle or aminoalkyl which is
 unsubstituted or substituted with hydroxy in the alkyl
 moiety, in the free form or in the form of an acid addition
 salt or a quaternary ammonium salt).

10 Among the pleuromutilin derivatives, particularly
 preferred is 14-deoxy-14-[(2-D-valylamino-1,1-dimethylethyl)-
 thioacetoxymutilin (designated as the Derivative No. 1
 hereinafter).

Tiamulin (R_1 = vinyl, R_2 = H, R_3 = H, R_4 = diethylamino
 15 in the above formula) and derivatives thereof in the form of
 acid addition salts or quaternary ammonium salts are also
 preferred among the pleuromutilin derivatives. Among the
 tiamulin derivatives, particularly preferred is tiamulin
 hydrogenfumarate (designated as the Derivative No. 2
 20 hereinafter).

Though the Derivatives may be administered per se directly to fishes, they may preferably be administered in the form of powders, granules, wettable powders, solutions, tablets, etc., prepared by mixing the Derivatives with an
5 extender, a carrier, etc., such as starch, glucose, dextran, natural salt, calcium carbonate, kaolin, corn flour, soybean cake, fish meal, rice bran, lucerne meal, alcohols, fats and oils, cotton seed oil, sodium aspartate, sorbitan monostearate, Tween (trade name of a surfactant, from Atlas
10 Powder Co.), lactose, etc., or they may preferably be administered in combination with feed or living prey, as practiced in usual administration.

The amount of the Derivatives to be administered may vary depending on the kind of fishes, etc., but is suitably
15 about 0.1 to 1000 ppm and preferably 1 to 500 ppm in the normal situation.

[Example]

The present invention will be illustrated in more detail with reference to the following examples, but it should be
20 construed that the invention is in no way limited to these examples.

Example 1 (Solution)

The Derivative No. 1 (1 part), ethanol (89.9 parts)
25 and Sorpol (0.1 part, trade name of a surfactant, from Toho

Kagaku Kabushiki Kaisha) were homogeneously mixed to form a solution.

Example 2 (Dust or powder)

5 The Derivative No. 1 (2 parts), sodium chloride (79.9 parts) and Sorpol (0.1 part, trade name of a surfactant, Toho Chemical Co., Ltd.) were homogeneously mixed and pulverized to give a dust (a powder).

Example 3 (Feed)

10 The Derivative No.1 (10 parts) was dissolved in acetone (90 parts) to form a solution (100 parts). The solution was sprinkled over young fish feed (999900 parts, from Nippon Haigo Shiryo Kabushiki Kaisha) and the solution and the feed were homogeneously mixed while acetone was removed to give feed containing 10 ppm of the Derivative No. 15 1.

Example 4 (Feed)

The Derivative No. 1 (0.2 part) and a soybean cake powder (99.8 parts) were homogeneously mixed and the mixture was pulverized to give a powder mixture (100 parts). The 20 powder mixture was homogeneously mixed with young fish feed (9900 parts, from Nippon Haigo Shiryo Kabushiki Kaisha) to give feed containing 20 ppm of the Derivative No. 1.

[Effects]

The preventing and treating effects of the 25 Derivative No. 1 on bacterial diseases of fishes are now

particularly illustrated by means of the following Test Examples.

Test Example 1

The solution was dissolved in methanol or distilled
5 water. The resultant solution was diluted stepwise to have
concentrations of the Derivative No. 1 of 100 µg/ml, 50
µg/ml, 5 µg/ml and 1 µg/ml, and assayed for antibacterial
activity at each of the concentrations. That is to say, a
paper disk 8 mm in diameter was impregnated with one of the
10 diluted solutions, air dried in a clean bench for 1 hour,
transferred on an agar medium (*2) inoculated with a fish
pathogen (0.1 ml) (*1) by means of Conradi and incubated
(*3). The antibacterial activity was judged by the presence
or absence of an inhibiting circle.

- 15 *1) amount of inoculated bacteria: 10^8 CUF
 *2) medium for judgement: medium for a sensitivity disk
 *3) conditions for incubation: 26.5°C for 40 hours

The results are shown in Table 1 wherein +++
designates very effective, ++ designates intermediately
20 effective, + designates slightly effective and - designates
ineffective.

Table 1

	Compound	Dose (µg/ml)	Streptococcus	Renibacterium
			sp. *	Salmoninarum
5	Derivative	100	+++	+++
	No. 1	50	+++	+++
		5	+++	+++
		1	+++	+++
10	Comparative	100	+++	-
	compound	50	++	-
	No. 3	5	-	-
		1	-	-
15	Comparative	100	+++	++
	compound	50	+++	+
	No. 4	5	+++	-
		1	+++	-

Note) No. 1: 14-deoxy-14-[(2-D-valylamino-1,1-dimethylethyl)-thioacetoxymutilin

20 No. 3: ampicillin

No. 4: erythromycin

*: pathogen for streptococcosis of young yellowtail

Test Example 2: Effect on streptococcosis of young yellowtail

5 Immediately thereafter, the infected fishes were placed
in a water bath (100 liter) containing 1, 5 or 100 ppm of the
Derivative No. 1 (as described above) and maintained in the
bath for 10 days. After this period, all the fishes of the
medical bath-treated group survived without onset of disease,
10 whereas all the fishes of the untreated infected control
group died within 2 weeks from infection, presenting necrosis
at the infected site.

15 Groups of salmon (one group consisting of 20
fishes) were combined with 10 salmon per group suffering
from bacterial kidney disease. The fishes were kept with
feed containing 2 ppm of the Derivative No. 1 (as described
above) for 15 days. After one month, the survival rate was
20 measured. The group treated with the Derivative No. 1 showed
a survival rate of 80%, whereas the untreated group showed a
fatality rate of 85%.

The above results have proved that the Derivatives are effective as agents for preventing or treating bacterial diseases of fishes.

Example 5 (Solution)

The Derivative No. 2 (1 part), ethanol (89.9 parts) and Sorpol (0.1 part, trade name of a surfactant, from Toho Kagaku Kabushiki Kaisha) were homogeneously mixed to form a solution.

Example 6 (Dust or powder)

The Derivative No. 2 (2 parts), sodium chloride (79.9 parts) and Sorpol (0.1 part, trade name of a surfactant, Toho Chemical Co., Ltd.) were homogeneously mixed and pulverized to give a dust (a powder).

Example 7 (Feed)

The Derivative No. 2 (10 parts) was dissolved in acetone (90 parts) to form a solution (100 parts). The solution was sprinkled over young fish feed (999900 parts, from Nippon Haigo Shiryo Kabushiki Kaisha) and the solution and the feed were homogeneously mixed while acetone was removed to give feed containing 10 ppm of the Derivative No. 2.

Example 8 (Feed)

The Derivative No. 2 (0.2 part) and a soybean cake powder (99.8 parts) were homogeneously mixed and the mixture was pulverized to give a powder mixture (100 parts). The powder mixture was homogeneously mixed with young fish feed (9900 parts, from Nippon Haigo Shiryo Kabushiki Kaisha) to give feed containing 20 ppm of the Derivative No. 2.

[Effects]

The preventing and treating effects of the Derivative No. 2 on bacterial diseases of fishes are now particularly illustrated by means of the following Test

5 Examples.

Test Example 4

The solution was dissolved in methanol or distilled water. The resultant solution was diluted stepwise to have concentrations of the Derivative No. 2 of 100 µg/ml, 50
10 µg/ml, 5 µg/ml and 1 µg/ml, and assayed for antibacterial activity at each of the concentrations. That is to say, a paper disk 8 mm in diameter was impregnated with one of the diluted solutions, air dried in a clean bench for 1 hour, transferred on an agar medium (*2) inoculated with a fish
15 pathogen (0.1 ml) (*1) by means of Conradi and incubated (*3). The antibacterial activity was judged by the presence or absence of an inhibiting circle.

*1) amount of inoculated bacteria: 10^8 CUF

*2) medium for judgement: medium for a sensitivity disk

20 *3) conditions for incubation: 26.5°C for 40 hours

The results are shown in Table 2 wherein +++ designates very effective, ++ designates intermediately effective, + designates slightly effective and - designates ineffective.

Table 2

	Compound	Dose (µg/ml)	Streptococcus	Renibacterium
			sp. *	Salmoninarum
5	Derivative	100	+++	+++
	No. 2	50	+++	+++
		5	+++	++
		1	++	+
10	Comparative	100	+++	-
	compound	50	++	-
	No. 5	5	-	-
		1	-	-
15	Comparative	100	+++	++
	compound	50	+++	+
	No. 6	5	+++	-
		1	+++	-

Note) No. 2: tiamulin hydrogenfumarate

No. 5: ampicillin.

20 No. 6: erythromycin

*: pathogen for streptococcosis of young yellowtail

Test Example 5: Effect on streptococcosis of young

yellowtail

Groups of young yellowtail (one group consisting of 10
25 fishes) were intramuscularly challenged by living bacteria

(10^7 /fish) of streptococcus sp. (the pathogen for streptococcosis of young yellowtail).

Immediately thereafter, the infected fishes were placed in a water bath (100 liter) containing 5 or 100 ppm of the
5 Derivative No. 2 (as described above) and maintained in the bath for 10 days. After this period, all the fishes of the medical bath-treated group survived without onset of disease, whereas all the fishes of the untreated infected control group died within 2 weeks from infection, presenting necrosis
10 at the infected site.

Test Example 6: Effect on bacterial kidney disease of salmon

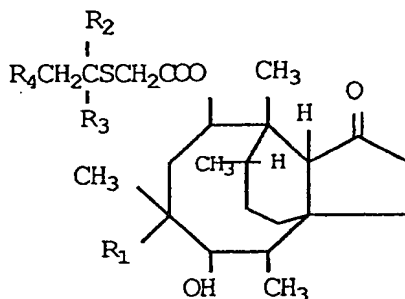
Groups of salmons (one group consisting of 20 fishes) were combined with 10 salmons per group suffering
15 from bacterial kidney disease. The fishes were kept with feed containing 2 ppm of the Derivative No. 2 (as described above) for 15 days. After one month, the survival rate was measured. The group treated with the Derivative No. 2 showed a survival rate of 70%, whereas the untreated group showed a
20 fatality rate of 85%.

The above results have proved that the Derivatives are effective as agents for preventing or treating bacterial diseases of fishes.

Claims;

(1) An agent for the prevention or treatment of bacterial diseases of fishes comprising, as an active ingredient, a pleuromutilin derivative of the formula:

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wherein R_1 is ethyl or vinyl, R_2 is hydrogen or methyl, R_3 is hydrogen or methyl, and R_4 is dialkylamino or R_5 -CONH- (R_5 being 5-membered saturated heterocycle or aminoalkyl which is unsubstituted or substituted with hydroxy in the alkyl moiety, in the free form or in the form of an acid addition salt or a quaternary ammonium salt).

(2) The agent for prevention or treatment according to claim 1, wherein said bacterial diseases of fishes are streptococcosis or bacterial kidney disease.

(3) The agent for prevention or treatment according to claim 2, wherein said pleuromutilin derivative is 14-deoxy-14-[(2-D-valylamino-1,1-dimethylethyl)thioacetoxymutilin.

(4) The agent for prevention or treatment according to claim 2, wherein said pleuromutilin derivative is tiamulin or a tiamulin derivative in the form of an acid addition salt or a quaternary ammonium salt.

- 5 (5) The agent for prevention or treatment according to claim 4, wherein said tiamulin derivative is tiamulin hydrogenfumarate.